

University of Groningen

A simple method for serially sectioning small specimens of hard tissue

Luiten, P.G.M.

Published in:
Stain technology

DOI:
[10.3109/10520297609116732](https://doi.org/10.3109/10520297609116732)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1976

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Luiten, P. G. M. (1976). A simple method for serially sectioning small specimens of hard tissue. *Stain technology*, 51(6), 309-310. <https://doi.org/10.3109/10520297609116732>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

REFERENCES

- Committee on Standardized Genetic Nomenclature for Mice. 1972. Standard karyotype of the mouse, *Mus musculus*. J. Hered. 63: 69-72.
- Nesbitt, M. N. and Francke, U. 1973. A system of nomenclature for band patterns of mouse chromosomes. Chromosoma 41: 145-158.
- Seabright, M. 1971. A rapid banding technique for human chromosomes. Lancet 2: 971-972.
- Wroblewska, J. and Dyban, A. P. 1969. Chromosome preparations from mouse embryos during early organogenesis: dissociation after fixation, followed by air drying. Stain Technol. 44: 147-150.

A SIMPLE METHOD FOR SERIALY SECTIONING SMALL SPECIMENS OF HARD TISSUE

P. G. M. LUITEN, *Zoological Laboratory, State University of Groningen, Haren, The Netherlands*

Serial sectioning of hard, fragile tissues such as arthropod cuticle usually offers problems. To overcome these often requires time-consuming techniques such as celloidin embedding or special methods that suit the specific demands of the tissue under study. The following technique is universal in its application and can be adapted to the requirements of specific tissues during sectioning. The principle is to embed the tissue in a relatively soft epoxy resin (Butler 1971, Luft 1961) and section in the cryostat. By changing the temperature in the freezing compartment the hardness of the epoxy resin can be adapted to the hardness of the tissue. The sections produced are of excellent quality, completely flat with a perfect attachment of the resin to the tissue. This technique guarantees a minimum of tissue distortion and prevents loss of fine and fragile appendages. Because of the difficulty of staining epoxy embedded sections the method is especially suitable for previously stained tissue. It is presumably adaptable to "open" type freezing microtomes as well. In this laboratory the method has successfully been applied to Golgi-impregnated teleostean brains, intracellularly stained nervous elements in fly heads, to blowfly proboscis, cricket abdomen and similar structures.

Procedure

1. Rinse briefly fixed tissue specimens with maximum dimensions of 10 mm in several changes of distilled water.

2. Dehydrate tissue in an ascending acetone series, 50%, 80%, 90%, and 3 changes of 100%. Twenty minutes for each step is a safe minimum for small pieces of tissue. Times should be adapted to the size of the tissue.

3. Immerse overnight in a 1:1 epoxy-acetone mixture. The epoxy resin is prepared as follows:

Araldite	26 ml
Epon (Epikote 812)	34 ml
Dodecenyl succinic anhydride (DDSA)	60 ml
Tridimethylaminomethyl phenol (DMP-30)	1.2 ml
Dibutyl phthalate	7.5 ml

4. Transfer the tissue into undiluted epoxy resin as above for about 6 hours at room temperature.

5. Bring the tissue into fresh resin in rectangular plastic cups of about $3 \times 3 \times 3$ cm (*e.g.* as for ice cubes). The inside of the cups should be treated with "Trennlack" (F86, Goldschmidt Chem. Co., Hamburg, Germany) for easy removal of the hardened blocks. Harden the epoxy resin for about 48 hours at 80 C.

6. The hardened blocks may be easily trimmed with a razor blade. Remove as much embedding medium as possible.

7. Attach the trimmed block to a flat tissue holder with cyanoacrylate adhesive and mount in the cryostat. Vary the temperature according to the hardness of the tissue and the required thickness of the sections; -10 C is suitable for $40\text{ }\mu\text{m}$ sections of Golgi impregnated brains, or -25 C for $10\text{ }\mu\text{m}$ sections of insect tissue.

8. Cut sections and bring to absolute alcohol for a few seconds, then mount on slides with the following mixture:

100% Alcohol	30 cc
Epoxy resin	8 cc
Acetone	2 cc

First mix the epoxy with the acetone, then add the alcohol. Thick sections of $20\text{ }\mu\text{m}$ or more can be mounted with undiluted resin. In such cases floating the sections in absolute alcohol should be omitted. In both mounting procedures spread a film of the resin on the slide with for example the back of the little finger. Transfer the sections to the slide; they will stick to the epoxy film very well and will not curl. Work out any air bubbles with a fine brush.

9. Apply a small drop of resin to the sections and cover with a coverslip. Harden overnight at 80 C.

REFERENCES

- Butler, R. 1971. Very rapid selective silver (Golgi) impregnations and embedding of invertebrate nervous tissue. *Brain Res.* 33: 540-544.
- Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9: 409-414.